XXXVII. NOTE ON A QUANTITATIVE SKATOLE COLOUR REACTION FOR FRUCTOSE

By RONALD CHARLES JORDAN and JOHN PRYDE

From The Physiology Institute, Cardiff

(*Received 7 December 1937*)

Various methods have been described for the detection and estimation in physiological fluids of small amounts of fructose in the presence of other carbohydrates. The Seliwanoff reaction and its quantitative modifications [Folin & Berglund, 1922; Roe, 1934] are well known. The blue coloration formed from fructose and diphenylamine in the presence of concentrated hydrochloric acid at 100° [Ihl & Pechmann, 1885] has been judged to be superior to the Seliwanoff reaction for quantitative use; it has been developed by van Creveld [1928], Kronenberger & Radt [1927], Radt [1928], Corley [1929], Oppel [1930], Steinitz & von Riesen [1932] and Patterson [1935] for this purpose. Another possible method described by Scott [1935] involves the use of bile salts in place of diphenylamine. Inasmuch as few investigators seem to have adopted without modification the methods of previous workers, it might be inferred that a satisfactory method is still to be sought. The main part of the investigation described in this note was carried out in ignorance of the diphenylamine and bile salt methods, but comparisons between these and our skatole method indicate that the latter is based upon a reaction of markedly greater sensitivity.

The skatole colour reaction originated from the chance observation of the development of an intense purple colour when a dried milk preparation was inadvertently heated by a student with conc. HCl. Curiously enough and quite independently a similar colour had been observed and noted for investigation a few days previously when ovomucoid had been heated with HCl. Attention was immediately directed to the possibility that tryptophan was a participant in the reaction, probably, in view of the similarity of the colour produced to that of the well-known glyoxylic acid test for tryptophan, by condensation with a carbohydrate derivative. It was found that tryptophan when heated in the presence of HCl with lactose, or with any hexose or hexose derivative, yielded the purple product. Several amino-acids other than tryptophan were investigated with negative results. It was sought to replace tryptophan with other compounds containing an indole nucleus. Various substituted indoles (e.g. indole-2-carboxylic acid and some of its derivatives) were used. These produced no colour on being warmed with HCl in the presence of a hexose. Indole and indole-6-carboxylic acid, in which the 2-position is unsubstituted, produced orange-yellow colours. On the other hand, when skatole was used in place of tryptophan, the colour reaction was intensified to a remarkable degree. It has not been possible to confirm the observation [*vide* Plimmer, 1933] that skatole alone when dissolved in conc. HCl yields a violet-coloured solution. In this medium, after warming to 80°, recrystallized skatole remains completely colour-free for a considerable time. Traces of carbohydrate impurity produce varying degrees of colour.

The development of the intense purple colour in the presence of skatole and HCl at 80–100° was found to be positive for all hexoses, substituted hexoses, and their derivatives examined (24 in number). Furthermore, the reaction is very much more readily obtained with fructose and its derivatives than with aldo-

hexoses, so much so that, on heating at 40° for 15 min., fructose, its derivatives (including hexosediphosphate from yeast fermentation) and sorbose alone yield a colour. Pentoses produce an orange-brown colour with skatole and HCl. Of the large number of hydroxylic, ketonic and aldehydic substances examined additionally to the sugars already mentioned, only the methylpentose, rhamnose, and glyoxylic acid give colours at all similar to those formed in the presence of hexoses. Rhamnose and skatole give with HCl a characteristic ruby-red colour distinct from the "permanganate" purple of the hexoses, whereas glyoxylic acid produces a colour with a much higher blue content.

Carried out at 80° or higher the skatole colour reaction provides a highly sensitive qualitative or quantitative test for hexoses generally; using the reaction developed at 40° we have found it possible to detect and estimate fructose with accuracy in the presence of a large excess of glucose. Using the test in a qualitative way procedure may be as follows.

Approximately 5–10 mg. of substance (according to the probable sugar content) are placed in a clean, dry boiling-tube (50 ml. capacity) with roughly 10 mg. recrystallized skatole and 10 ml. conc. A.R. HCl. The tube is then placed in a water-bath at 38–40° for 30 min. If an intense "permanganate" purple colour develops under these conditions fructose (or other ketohexoses or complexes containing these) is present in the substance under test. In the absence of a ketohexose the test is continued by placing the tube in a water-bath at 80° for 15 min. when the presence of an aldohexose is demonstrated by the development of the intense purple colour. The subsequent addition of absolute alcohol intensifies the reaction by dissolving the excess skatole which absorbs some of the coloured product from aqueous acid solution. Aliquots of aqueous solutions may be tested in the same manner.

For quantitative purposes it is not claimed that methods based on the skatole reaction will be preferred under all circumstances to those methods previously described. Nevertheless, its greater sensitivity affords a possible basis for the development of a highly accurate and selective method for the determination of fructose in the presence of other sugars as, for instance, in blood and other physiological fluids and tissues. It is not proposed to give here full experimental details concerning our own numerous investigations of the applicability of the skatole colorimetric method. These followed lines which will be obvious to users of similar methods. In preliminary investigations accurate determinations of fructose added to plasma were made with ease in the presence of considerable amounts of added glucose, as is shown in Table I. In these experiments deproteinization was effected by the addition of trichloroacetic acid and colorimetric comparisons were made with a Leitz compensating colorimeter in solutions containing 50 % ethyl alcohol.

Table I. *Determination of fructose added to plasma*

Fructose added mg./100 ml.	Glucose added mg./100 ml.	Fructose found mg./100 ml.
20·0	—	20·0
40·0	—	40·1
60·0	—	61·0
20·0	100	19·2
30·0	100	29·2
40·0	120	41·3
40·0	40	41·7

The expenses of this investigation were in part defrayed by a grant from the Medical Research Council.

REFERENCES

Corley (1929). *J. biol. Chem.* **81**, 81.
van Creveld (1928). *Arch. néerl. Physiol.* **13**, 521.
Folin & Berglund (1922). *J. biol. Chem.* **51**, 209.
Ihl & Pechmann (1885). *Chemikerztg.* **25**, 451.
Kronenberger & Radt (1927). *Biochem. Z.* **190**, 161.
Oppel (1930). *Biochem. Z.* **229**, 85.
Patterson (1935). *Biochem. J.* **29**, 1398.
Plimmer (1933). Organic and Biochemistry. Longman and Green, Aberdeen.
Radt (1928). *Biochem. Z.* **198**, 195.
Roe (1934). *J. biol. Chem.* **107**, 16.
Scott (1935). *Biochem. J.* **29**, 1012.
Steinitz & von Riesen (1932). *Biochem. Z.* **252**, 201.